Lipoprotein-associated phospholipase A_2: a potential new risk factor for coronary artery disease and a therapeutic target

Colin H Macphee

The recognition that atherosclerosis represents an inflammatory disease has begun to shift interest towards novel therapies that could specifically target the underlying inflammatory component of atherogenesis. Like low-density lipoprotein, an ideal new drug target would be a modifiable plasma risk factor that not only reflects the ongoing inflammatory process but also actively promotes it. Lipoprotein-associated phospholipase A_2, also known as platelet-activating factor acetylhydrolase, is a new risk factor that may have the potential to fulfil these requirements.

Addresses
Department of Vascular Biology, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park North, Third Avenue, The Pinnacles, Harlow, Essex CM19 5AW, UK;
e-mail: Colin_H_Macphee@sbphrd.com

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Abbreviations

CHD	coronary heart disease
CRP	C-reactive protein
Il-6	interleukin-6
LDL	low-density lipoprotein
Lp-PLA_2	lipoprotein-associated phospholipase A_2
LPS	lipopolysaccharide
lyso-PC	lysophosphatidylcholine
oxLDL	oxidised low-density lipoprotein
WOSCOPS	West of Scotland Coronary Prevention Study

Introduction

Within the past decade there has been a general acceptance that atherosclerosis is primarily an inflammatory disease, although limited to the arterial intima [1••]. The disease process is thought to be a response to a damaging insult, which is probably chemical rather than physical. The major candidates for this action are thought to be accumulated oxidised low-density lipoprotein (oxLDL), and/or an infectious agent possibly working in concert [2••,3,4••]. At all stages of atherogenesis, arterial lesions contain monocytes, macrophages and T-lymphocytes together with a dysfunctional endothelium.

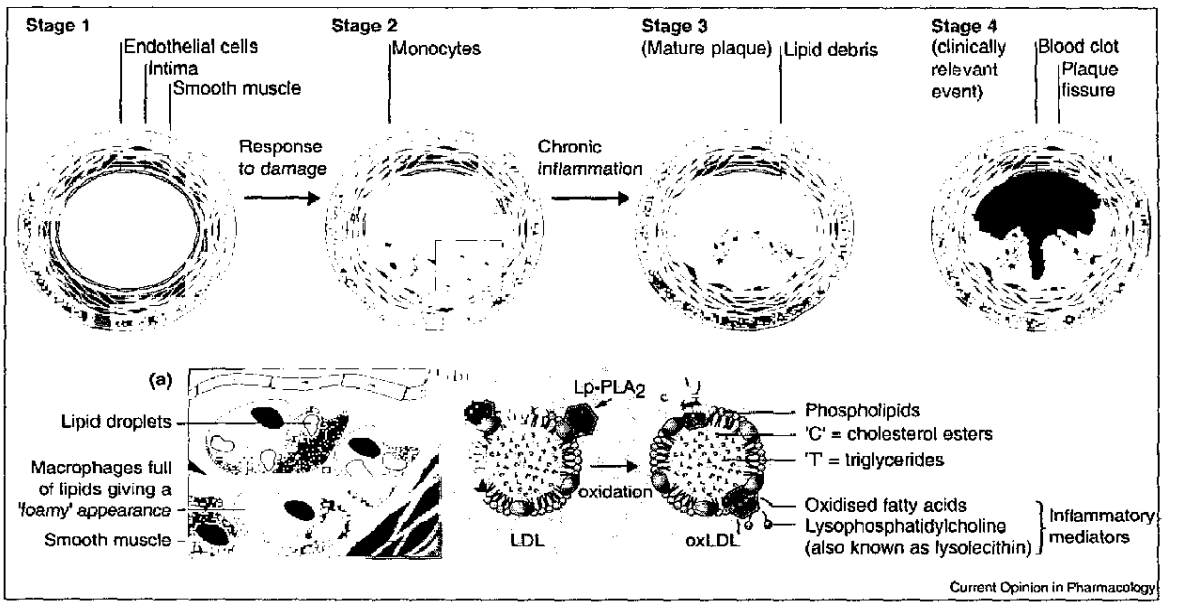
Monocytes in particular appear to play an important role in the pathophysiology of acute coronary syndromes. They are attracted to the damaged artery and migrate to the intima where they differentiate into macrophages, which then devour oxLDL in an unregulated method, becoming fat-laden and taking on a characteristic 'foamy' appearance [5]. Meanwhile, the inflammatory process remains unchecked. More cells are attracted to the site and more oxLDL accumulates within the artery wall. Over time this process leads to the formation of a mature yellowish plaque. These more advanced plaques consist of a fibrous cap covering a core of dead and dying cells and other debris including cholesterol and calcium salts. This core is surrounded by inflammatory cells that are dominated by monocyte-derived macrophages. These advanced atherosclerotic lesions are susceptible to fissure or rupture, leading to thrombosis and life-threatening acute myocardial infarction. Plaque disruption is now viewed as a reflection of enhanced inflammatory activity within the plaque. Pathological studies have clearly demonstrated that advanced plaques containing high densities of macrophages, particularly near the cap, are most prone to fissure [6••]. These clinically relevant lesions are often referred to as 'vulnerable' plaques.

The recognition that atherosclerosis is an inflammatory disease has led to the search for plasma markers of inflammation that could accurately predict susceptibility to coronary heart disease (CHD). In addition, new therapeutic approaches are being postulated to specifically reduce the inflammatory response and, thus, stabilise atherosclerotic plaques. An ideal new drug target, therefore, would be based upon a modifiable risk factor for CHD that does not simply reflect the ongoing inflammatory process but that actively promotes it. Given the current understanding of the pathogenesis of atherosclerosis, this target would participate at all the critical stages: oxidation of low-density lipoprotein (LDL), infiltration and accumulation of inflammatory cells as well as modulation through infection. The focus of this review is lipoprotein-associated phospholipase A_2 (Lp-PLA_2), which satisfies many of these criteria and, therefore, represents a new target for the treatment of CHD.

Indices of inflammation: marker or risk factor?

Serum/plasma measurements of inflammation-associated proteins have recently been identified as potential solutions for detecting high-risk individuals with vulnerable plaques [7••,8]. Among the proteins measured are high-sensitivity C-reactive protein (CRP), interleukin-6 (Il-6), fibrinogen, soluble intercellular adhesion molecule type 1, and type II secretory phospholipase A_2, all of which are associated with an increased risk of CHD. It is not clear whether these molecules play a causative role or simply act as markers of, for example, the acute phase reaction [9•]. Several recent studies have demonstrated a strong link between baseline elevations of CRP and future risk of coronary events; however, CRP is viewed as a relatively nonspecific marker of low-grade systemic inflammation because the synthesis of CRP by the liver is largely regulated by Il-6 [8]. CRP, therefore, represents a marker of IL-6 release and, hence, of interleukin-1 release. Thus, such markers of inflammation appear to reflect the consequence of elevated levels of

Figure 1



Atherosclerosis, LDL and Lp-PLA$_2$. Stage 1: atherosclerosis is thought to start with damage to the endothelial layer that lines the inner surface of the artery, possibly caused by the action of accumulated oxLDL. Stage 2: monocytes are attracted to the artery and migrate to the intima where they transform into macrophages. At the same time, LDL collects in the artery and becomes oxidised. Macrophages devour oxLDL and take on a characteristic 'foamy' appearance (a). The action of Lp-PLA2 potentiates this inflammatory process: (b) the enzyme Lp-PLA$_2$ clings to LDL but remains inactive until LDL undergoes oxidation within the plaque environment. The phospholipid component of oxLDL immediately becomes susceptible to enzymatic attack by Lp-PLA$_2$ generating substantial concentrations of two new lipid products – oxidised fatty acid and lysophosphatidylcholine – both highly effective inflammatory mediators capable of driving the atherogenic process. Stage 3 (mature plaque): the inflammatory process remains unchecked. More cells are attracted to the site and more oxLDL accumulates within the vessel wall. Stage 4 (clinically relevant event): advanced atherosclerotic plaques are susceptible to fissure or rupture, leading to the immediate formation of blood clots that can cause vascular occlusion and ultimately death.

pro-inflammatory cytokines [7••]. A similar situation exists for type II secretory phospholipase A_2, an enzyme with potential to participate in atherogenesis, because plasma levels are highly and significantly correlated with CRP levels [10]. Thus, although there are now several downstream indicators of inflammation, none of these represent unequivocal causative candidates.

Plasma levels of the enzyme Lp-PLA$_2$ have recently been shown to be a new and independent measure of CHD risk in a nested case-control study involving individuals who participated in the West of Scotland Coronary Prevention Study (WOSCOPS; [11••]). Unlike most epidemiological studies, several different markers of inflammation were evaluated together in order to gauge their overall relative contribution. Subsequent analysis confirmed CRP and fibrinogen as predictors of CHD risk in WOSCOPS, but also demonstrated that both, together with white blood cell count, were substantially compromised in multivariant analyses. This observation would tend to confirm them as downstream markers of inflammation; however, the same analysis identified Lp-PLA$_2$ levels as a strong and independent predictor of clinical events not influenced by CRP, fibrinogen, white blood cell count or even standard lipid parameters, including LDL with which it associates. Although the epidemiology for Lp-PLA$_2$ needs to be extended to different populations, the unique risk profile of Lp-PLA$_2$ suggests that it could be a causative risk factor. To consider this possibility fully, we review the functions of this enzyme with particular reference to the aetiology of atherogenesis.

Lipoprotein-associated phospholipase A_2: a causative agent?

Lp-PLA$_2$ is a secreted calcium-independent member of the phospholipase A_2 superfamily produced mainly, if not exclusively, by monocytes, macrophages, T-lymphocytes and mast cells [12,13]. Interestingly, it is exactly this leukocyte infiltrate that is intimately involved in atherosclerosis [1••,14]. Lp-PLA$_2$ activity has been shown to be upregulated in atherosclerotic lesions, especially in more complex plaques, co-localising with macrophages [15]. The positive association of plasma Lp-PLA$_2$ with CHD, however, is not fully explained by this enhanced expression; for example, elevated CRP levels that reflect ongoing inflammation were not identified in WOSCOPS as a strong independent

risk factor [11••]. Furthermore, Lp-PLA$_2$ does play an active role during the oxidation of LDL [16••]. Lp-PLA$_2$ levels are not, therefore, a reflection of atherosclerotic burden, and are clearly differentiated from other inflammatory components of atherosclerosis.

Generation of lysophosphatidylcholine during oxidation of low-density protein

Approximately 80% of Lp-PLA$_2$ in plasma resides on LDL, with the remainder distributed across high-density lipoprotein and very low-density lipoprotein [17]. Such an association is highly significant because the deposition of LDL, particularly in an oxidised form, within the artery wall is still viewed as the single most important stage of atherosclerosis. The importance of oxidised LDL has emerged as a result of the numerous reports describing a pro-inflammatory activity when compared with native, unmodified LDL [18]. Although oxidatively damaged LDL has been detected in plaques, the mechanisms by which lipoproteins become oxidised *in vivo* remain largely unknown. Irrespective of the mechanism, however, one of the earliest consequences of LDL oxidation is the rapid degradation of oxidised phosphatidylcholines generating substantial quantities of lysophosphatidylcholine (lyso-PC) and free oxidised fatty acids. The cleavage of oxidised phosphatidylcholines within modified LDL-particles is carried out solely by Lp-PLA$_2$ [16••]; Lp-PLA$_2$ remains latent until LDL undergoes oxidative damage. This action is highly significant because both of these new lipid products are highly effective inflammatory mediators capable of attracting monocytes [16••] and exacerbating the atherogenic process (Figure 1).

The biological actions of the free oxidised fatty acids released by Lp-PLA$_2$ are not fully defined because their structure has not yet been characterised. One would predict, however, that micromolar concentrations of these fatty acids from oxLDL would not be biologically inert. In contrast, the number of reports ascribing a pro-inflammatory and pro-atherogenic role for lyso-PC has increased exponentially during the 1990s (C Macphee, unpublished data). Relevant pro-atherogenic activities of lyso-PC include impairment of endothelium-dependent relaxations, induction of vascular cell and intercellular adhesion molecules, chemoattractant for monocytes and T-lymphocytes, suppressed production and release of endothelium-derived nitric oxide, mitogenic for macrophages, inhibition of macrophage migration, cytotoxic at concentrations greater than 30–50 micromolar, upregulation of cytokine and CD40 ligand expression by T cells, stimulation of the release of arachidonic acid from endothelial cells, induction of monocyte chemoattractant protein-1 and genes for growth factors and involvement in the antigenicity of oxLDL. The endothelium, in particular, appears to be sensitive to elevations in lyso-PC concentration, which is most relevant to atherogenesis because a normal endothelium fulfils a number of protective functions including maintaining a non-thrombogenic surface. Further evidence implicating lyso-PC in the pathological states of vascular endothelium comes from recent studies showing that lyso-PC can inhibit endothelial cell migration and proliferation [19] as well as stimulate superoxide production [20].

Lyso-PC is an amphipathic molecule that is freely soluble in aqueous solution and is able to adversely affect all of the relevant cell types involved in the pathophysiology of atherosclerosis. The observation that lyso-PC levels build up within lesions increases the likelihood that it can actively participate in driving the disease process. Finally, although it is well known that esterified and non-esterified cholesterol accumulate in atherosclerotic plaques, there is also a concomitant decrease in phosphatidylcholine levels, potentially through Lp-PLA$_2$ action. Taken together these effects could potentially destabilise the atherosclerotic plaque by making the lipid core less solid, which might favour fissuring and rupture.

Infection, low-density lipoprotein subfractions and novel inhibitors

A high incidence of CHD occurs in patients with *Chlamydia pneumoniae* and cytomegalovirus infections, and these microorganisms have been detected in atherosclerotic plaques. These and other epidemiological studies have suggested a link between atherosclerosis and inflammation. Infection is accompanied by a systemic host response known as the acute-phase response that can be reproduced by the administration of endotoxin (i.e. lipopolysaccharide; LPS) [4••]. LPS administration decreases high-density lipoprotein, and increases triglycerides and the very atherogenic small dense LDL. Very recently it has also been demonstrated in experimental animals that LPS substantially increases the cellular expression of Lp-PLA$_2$ and, as a consequence, its plasma activity [21,22]. Concomitant with this increased expression was enhanced LDL oxidation and a massive increase in the lyso-PC content of circulating LDL [23]. The conclusion made by the authors was that the increase in lyso-PC content was secondary to the increase in plasma Lp-PLA$_2$ activity. As such, the enhanced contribution of Lp-PLA$_2$ that occurs during infection could represent a mechanism that promotes atherosclerosis in patients with chronic infections. The atherogenic lipoprotein profile produced by LPS administration may also contribute to the mechanism by which Lp-PLA$_2$ can augment atherogenesis because it is known that the enzyme is enriched in LDL subfractions that have a prolonged plasma half-life, which includes the very atherogenic small dense LDL.

Novel and potent inhibitors of Lp-PLA$_2$ have recently been described [24]. These have been used to evaluate and confirm the role of Lp-PLA$_2$ during the *in vitro* oxidation of LDL as well as determining whether inhibition of Lp-PLA$_2$ *in vivo* modifies disease progression. Importantly, in addition to abolishing the enhanced monocyte chemoattractant activity of oxidised LDL *in vitro*

[16••], inhibition of Lp-PLA_2 in the Watanabe heritable hyperlipidaemic rabbit leads to a significant reduction in plaque development [25•].

Lipoprotein-associated phospholipase A_2: an alternative view

Lp-PLA_2 is also known in the literature as plasma platelet-activating factor acetylhydrolase (PAF-AH). Although the enzyme has significantly broader substrate specificity than simply to cleave acetate from an ether phospholipid, it was first described as a plasma enzyme activity able to cleave PAF. Lp-PLA_2 is, therefore, a member of the PLA_2 superfamily able to recognise many different and complex polar phosphatidylcholines; however, owing to the ability of Lp-PLA_2 to hydrolyse PAF as well as structurally related oxidised phospholipids, the enzyme has been postulated to inhibit inflammatory processes including atherogenesis [26••]. This view is completely opposite to what the data from WOSCOPS is suggesting. Even though various potent PAF-receptor antagonists have failed to demonstrate anti-inflammatory efficacy in the clinic, this apparent paradox needs to be addressed scientifically.

Approximately 4% of the Japanese population are known to lack Lp-PLA_2 activity because they are homozygous for a common functional polymorphism within the gene. Knowing the predisposition of this genetic subgroup to CHD would help resolve the debate on the predominant activity of Lp-PLA_2: anti-inflammatory or pro-inflammatory. A number of clinical studies have been conducted on this missense mutation, but findings have been complex and ambiguous. The first, and largest to-date, reported a positive association in male patients but a negative association in females with diagnosed myocardial infarction [27,28]. Moreover, the positive association was only apparent in patient heterozygotes for the allele but not in homozygotes. A similar positive heterozygote-only association was found in patients with stroke [29], along with the intriguing caveat that patient heterozygotes had significantly higher plasma Lp-PLA_2 activity than control heterozygotes. The genetic observation did not, therefore, match the biochemical measurements. Clearly there is a requirement in future studies to focus on determining the frequency of homozygotes in controls versus patients because it will be this group alone that will provide an unequivocal answer as to whether Lp-PLA_2 associates with disease status. This need is highlighted by recent findings that investigated the association of the missense Lp-PLA_2 mutation in Japanese patients with asthma. One group found a positive association [30] whereas another did not [31].

Conclusions

This review has focused on the evidence supporting a role for LDL-associated Lp-PLA_2 in specifically driving the clinically relevant chronic inflammation that is so crucial to the pathophysiology of atherosclerosis. Evidence for a causative role of Lp-PLA_2 in atherogenesis comes from several sources:

1. Epidemiological: although Lp-PLA_2 is mainly LDL bound and, therefore, follows the association between LDL and enhanced atherosclerosis, very recent data has demonstrated that it is a strong and independent risk factor for CHD clinical events. This means it is the concentration of Lp-PLA_2 on LDL that appears critical.

2. Experimental: during the oxidation of LDL, Lp-PLA_2 is responsible for the large increase in concentration of the highly effective, multifunctional and toxic inflammatory mediator lyso-PC.

3. Pathophysiological: Lp-PLA_2 protein and its product, lyso-PC, are elevated in human plaques.

4. Pharmacological: inhibition of Lp-PLA_2 in the Watanabe heritable hyperlipidaemic rabbit model of atherosclerosis with a selective and potent small molecular weight inhibitor retarded disease progression.

In summary, although evidence is emerging to support a causative involvement of Lp-PLA_2 in atherogenesis, more epidemiological, pharmacological and genetic studies are required to confirm this role.

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